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(54) Title: IKAP PROTEINS, NUCLEIC ACIDS AND METHODS (57) Abstract The invention provides methods and compositions relating to IKAP proteins which regulate cellular signal transduction and transcriptional activation, and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKAP encoding nucleic acids or purified from human cells. The invention provides isolated IKAP hybridization probes and primers capable of specifically hybridizing with the disclosed IKAP genes, IKAP-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.		

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IKAP Proteins, Nucleic Acids and Methods

INTRODUCTION

5 Field of the Invention

The field of this invention is proteins involved in cell signal transduction.

Background

10 Cytokines trigger changes in gene expression by modifying the activity of otherwise latent transcription factors (Hill and Treisman, 1995). Nuclear factor κ B (NF- κ B) is a prominent example of how such an external stimulus is converted into an active transcription factor (Verma et al., 1995). The NF- κ B system is composed of homo- and heterodimers of members of the Rel family of related transcription factors that control the expression of numerous immune and inflammatory response genes as well as important viral
15 genes (Lenardo and Baltimore, 1989; Baeuerle and Henkel, 1994). The activity of NF- κ B transcription factors is regulated by their subcellular localization (Verma et al., 1995). In most cell types, NF- κ B is present as a heterodimer comprising of a 50 kDa and a 65 kDa subunit. This heterodimer is sequestered in the cytoplasm in association with I κ B α a member of the I κ B family of inhibitory proteins (Finco and Baldwin, 1995; Thanos and
20 Maniatis, 1995; Verma et al., 1995). I κ B α masks the nuclear localization signal of NF- κ B and thereby prevents NF- κ B nuclear translocation. Conversion of NF- κ B into an active transcription factor that translocates into the nucleus and binds to cognate DNA sequences requires the phosphorylation and subsequent ubiquitin-dependent degradation of I κ B α in the 26s proteasome. Signal-induced phosphorylation of I κ B α occurs at serines 32 and 36.
25 Mutation of one or both of these serines renders I κ B α resistant to ubiquitination and proteolytic degradation (Chen et al., 1995); DiDonato, 1996 #370. Roff, 1996 #397.

30 The pleiotropic cytokines tumor necrosis factor (TNF) and interleukin-1 (IL-1) are among the physiological inducers of I κ B phosphorylation and subsequent NF- κ B activation (Osborn et al., 1989; Beg et al., 1993). Although TNF and IL-1 initiate signaling cascades leading to NF- κ B activation via distinct families of cell-surface receptors (Smith et al., 1994; Dinarello, 1996), both pathways utilize members of the TNF receptor-associated factor (TRAF) family of adaptor proteins as signal transducers (Rothe et al., 1995; Hsu et al., 1996;

Cao et al., 1996b). TRAF proteins were originally found to associate directly with the cytoplasmic domains of several members of the TNF receptor family including the 75 kDa TNF receptor (TNFR2), CD40, CD30, and the lymphotoxin- β receptor (Rothe et al., 1994; Hu et al., 1994; Cheng et al., 1995; Mosialos et al., 1995; Song and Donner, 1995; Sato et al., 1995; Lee et al., 1996; Gedrich et al., 1996; Ansieau et al., 1996). In addition, TRAF proteins are recruited indirectly to the 55 kDa TNF receptor (TNFR1) by the adaptor protein TRADD (Hsu et al., 1996). Activation of NF- κ B by TNF requires TRAF2 (Rothe et al., 1995; Hsu et al., 1996). TRAF5 has also been implicated in NF- κ B activation by members of the TNF receptor family (Nakano et al., 1996); Ishida, 1996 #240. In contrast, TRAF6 participates in NF- κ B activation by IL-1 (Cao et al., 1996b). Upon IL-1 treatment, TRAF6 associates with IRAK, a serine-threonine kinase that binds to the IL-1 receptor complex (Cao et al., 1996a); Huang, 1997 #400.

The NF- κ B-inducing kinase (NIK) is a member of the MAP kinase kinase kinase (MAP3K) family that was identified as a TRAF2-interacting protein (Malinin et al., 1997). NIK activates NF- κ B when overexpressed, and kinase-inactive mutants of NIK comprising its TRAF2-interacting C-terminal domain (NIK₍₆₂₄₋₉₄₇₎) or lacking two crucial lysine residues in its kinase domain (NIK_(KK429-430AA)) behave as dominant-negative inhibitors that suppress TNF-, IL-1-, and TRAF2-induced NF- κ B activation (Malinin et al., 1997). Recently, NIK was found to associate with additional members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK also inhibited TRAF5- and TRAF6-induced NF- κ B activation, thus providing a unifying concept for NIK as a common mediator in the NF- κ B signaling cascades triggered by TNF and IL-1 downstream of TRAFs. Recently two NIK-interacting protein designated characterized as novel human kinase I κ B Kinases, IKK- α and IKK- β have been reported (Woronicz et al., 1997; Mercurio et al. 1997; Maniatis, 1997). Catalytically inactive mutants of IKK suppress NF- κ B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed IKK associates with endogenous I κ B α complex; and IKK phosphorylates I κ B α on serines 32 and 36.

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SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to isolated IKAP polypeptides, related nucleic acids, polypeptide domains thereof having IKAP-specific structure and activity and modulators of IKAP function, particularly NIK binding activity. IKAP polypeptides can regulate NF κ B activation and hence provide important regulators of cell function. The polypeptides may be produced recombinantly from transformed host cells from the subject IKAP polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated IKAP hybridization probes and primers capable of specifically hybridizing with the disclosed IKAP gene, IKAP-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for IKAP transcripts), therapy (e.g. IKAP inhibitors to inhibit TNF signal transduction) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating other transcriptional regulators, reagents for screening chemical libraries for lead pharmacological agents, etc.).

BRIEF DESCRIPTION OF THE FIGURE

Fig. 1. IKAP polypeptides activate NF κ B.

DETAILED DESCRIPTION OF THE INVENTION

The nucleotide sequence of a natural cDNA encoding a human IKAP polypeptide is shown as SEQ ID NO:1, and the full conceptual translate is shown as SEQ ID NO:2. The IKAP polypeptides of the invention include one or more functional domains of SEQ ID NO:2, which domains comprise at least 8, preferably at least 16, more preferably at least 32, most preferably at least 64 contiguous residues of SEQ ID NO:2 and have human IKAP-specific amino acid sequence and activity. IKAP domain specific activities include NIK-binding or binding inhibitory activity, NF κ B-binding or binding inhibitory activity and IKAP specific immunogenicity and/or antigenicity.

IKAP-specific activity or function may be determined by convenient *in vitro*, cell-based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g. gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the molecular interaction of an IKAP polypeptide with a binding target is evaluated. The binding target may be a natural intracellular binding target such as an IKAP binding target, a IKAP

regulating protein or other regulator that directly modulates IKAP activity or its localization; or non-natural binding target such a specific immune protein such as an antibody, or an IKAP specific agent such as those identified in screening assays such as described below. IKAP-binding specificity may assayed by binding equilibrium constants (usually at least about 10^7 M^{-1} , preferably at least about 10^8 M^{-1} , more preferably at least about 10^9 M^{-1}), by NF κ B reporter expression, by the ability of the subject polypeptide to function as negative mutants in IKAP-expressing cells, to elicit IKAP specific antibody in a heterologous host (e.g a rodent or rabbit), etc.

For example, deletion mutagenesis is used to defined functional IKAP domains which activate NF κ B expression or function as dominant/negative mutants in IKAP-mediated NF κ B activation assays. See, e.g. Table 1.

Table 1. Exemplary IKAP deletion mutants defining IKAP functional domains.

<u>Mutant</u>	<u>Sequence</u>	NF κ B	Dom/Neg
Δ N1	SEQ ID NO:2, residues 42-1332	+	-
Δ N2	SEQ ID NO:2, residues 142-1332	+	-
Δ N3	SEQ ID NO:2, residues 242-1332	+	-
Δ N4	SEQ ID NO:2, residues 342-1332	+	-
Δ N5	SEQ ID NO:2, residues 442-1332	+	-
Δ C1	SEQ ID NO:2, residues 1-923	-	+
Δ C2	SEQ ID NO:2, residues 1-441	-	
Δ C3	SEQ ID NO:2, residues 1-241	-	
Δ C4	SEQ ID NO:2, residues 1-241	-	

In a particular embodiment, the subject domains provide IKAP-specific antigens and/or immunogens, especially when coupled to carrier proteins. For example, peptides corresponding to IKAP- and human IKAP-specific domains are covalently coupled to keyhole limpet antigen (KLH) and the conjugate is emulsified in Freund's complete adjuvant. Laboratory rabbits are immunized according to conventional protocol and bled. The presence of IKAP-specific antibodies is assayed by solid phase immunosorbant assays using immobilized IKAP polypeptides of SEQ ID NO:2, see, e.g. Table 2.

Table 2. Immunogenic IKAP polypeptides eliciting IKAP-specific rabbit polyclonal antibody: IKAP polypeptide-KLH conjugates immunized per protocol described above.

	<u>IKAP Polypeptide Sequence</u>	<u>Immunogenicity</u>
	SEQ ID NO:2, residues 1-10	+++
	SEQ ID NO:2, residues 29-41	+++
5	SEQ ID NO:2, residues 75-87	+++
	SEQ ID NO:2, residues 92-109	+++
	SEQ ID NO:2, residues 132-141	+++
	SEQ ID NO:2, residues 192-205	+++
	SEQ ID NO:2, residues 258-269	+++
10	SEQ ID NO:2, residues 295-311	+++
	SEQ ID NO:2, residues 316-330	+++
	SEQ ID NO:2, residues 373-382	+++
	SEQ ID NO:2, residues 403-422	+++
	SEQ ID NO:2, residues 474-485	+++
15	SEQ ID NO:2, residues 561-576	+++
	SEQ ID NO:2, residues 683-697	+++
	SEQ ID NO:2, residues 768-777	+++
	SEQ ID NO:2, residues 798-813	+++
	SEQ ID NO:2, residues 882-894	+++
20	SEQ ID NO:2, residues 934-946	+++
	SEQ ID NO:2, residues 1054-1067	+++
	SEQ ID NO:2, residues 1181-1192	+++
	SEQ ID NO:2, residues 1273-1282	+++
	SEQ ID NO:2, residues 1283-1294	+++
25	SEQ ID NO:2, residues 1295-1312	+++
	SEQ ID NO:2, residues 1313-1332	+++

The claimed IKAP polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least

about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. The IKAP polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from mammalian, preferably human cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions. see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, *et al.* Cold Spring Harbor Laboratory). Current Protocols in Molecular Biology (Eds. Ausubel, *et al.*, Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

The invention provides binding agents specific to IKAP polypeptides, preferably the claimed IKAP polypeptides, including substrates, agonists, antagonists, natural intracellular binding targets, etc., methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. For example, specific binding agents are useful in a variety of diagnostic and therapeutic applications, especially where disease or disease prognosis is associated with improper utilization of a pathway involving the subject proteins, e.g. NF- κ B activation. Novel IKAP-specific binding agents include IKAP-specific receptors, such as somatically recombined polypeptide receptors like specific antibodies or T-cell antigen receptors (see, e.g. Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and other natural intracellular binding agents identified with assays such as one-, two- and three-hybrid screens, non-natural intracellular binding agents identified in screens of chemical libraries such as described below, etc. Agents of particular interest modulate IKAP function, e.g. IKAP-dependent transcriptional activation.

Accordingly, the invention provides methods for modulating signal transduction involving NF κ B in a cell comprising the step of modulating IKAP activity. The cell may reside in culture or in situ, i.e. within the natural host. For diagnostic uses, the inhibitors or other IKAP binding agents are frequently labeled, such as with fluorescent, radioactive, chemiluminescent, or other easily detectable molecules, either conjugated directly to the binding agent or conjugated to a probe specific for the binding agent. Exemplary inhibitors include nucleic acids encoding dominant/negative mutant forms of IKAP, as described above, etc.

The amino acid sequences of the disclosed IKAP polypeptides are used to back-translate IKAP polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or

used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural IKAP-encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). IKAP-encoding nucleic acids used in IKAP-expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with IKAP-modulated cell function, etc.

The invention also provides nucleic acid hybridization probes and replication / amplification primers having a IKAP cDNA specific sequence comprising at least 12, preferably at least 24, more preferably at least 36 and most preferably at least contiguous 96 bases of a strand of SEQ ID NO:1 sufficient to specifically hybridize with a second nucleic acid comprising the complementary strand of SEQ ID NO:1. Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO₄, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C.

Table 3. Exemplary IKAP nucleic acids which hybridize with a strand of SEQ ID NO:1 under Conditions I and/or II.

20	<u>IKAP Nucleic Acids</u>	<u>Hybridization</u>
	SEQ ID NO:1, nucleotides 1-47	+
	SEQ ID NO:1, nucleotides 58-99	+
	SEQ ID NO:1, nucleotides 95-138	+
	SEQ ID NO:1, nucleotides 181-220	+
25	SEQ ID NO:1, nucleotides 261-299	+
	SEQ ID NO:1, nucleotides 274-315	+
	SEQ ID NO:1, nucleotides 351-389	+
	SEQ ID NO:1, nucleotides 450-593	+
	SEQ ID NO:1, nucleotides 524-546	+
30	SEQ ID NO:1, nucleotides 561-608	+
	SEQ ID NO:1, nucleotides 689-727	+

	SEQ ID NO:1, nucleotides 808-837	+
	SEQ ID NO:1, nucleotides 938-1001	+
	SEQ ID NO:1, nucleotides 1205-1254	+
	SEQ ID NO:1, nucleotides 1855-1907	+
	SEQ ID NO:1, nucleotides 2910-2953	+
5	SEQ ID NO:1, nucleotides 3967-3999	+

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e. unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome. Recombinant nucleic acids comprising the nucleotide sequence of SEQ ID NO:1, or requisite fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, preferably fewer than 500 bp, which is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.: use in detecting the presence of IKAP genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional IKAP homologs and structural analogs. In diagnosis, IKAP hybridization probes find use in identifying wild-type and mutant IKAP alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic IKAP nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active IKAP.

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of a IKAP modulatable cellular function.

Generally, these screening methods involve assaying for compounds which modulate IKAP interaction with a natural IKAP binding target, such as NIK. A wide variety of assays for binding agents are provided including labeled *in vitro* protein-protein binding assays, immunoassays, cell based assays, etc. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials: for example, the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

In vitro binding assays employ a mixture of components including an IKAP polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g. a tag for detection or anchoring, etc. The assay mixtures comprise a natural intracellular IKAP binding target. While native full-length binding targets may be used, it is frequently preferred to use portions (e.g. peptides) thereof so long as the portion provides binding affinity and avidity to the subject IKAP polypeptide conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds: preferably small organic compounds and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the IKAP polypeptide specifically binds the cellular binding target, portion or analog with a reference binding affinity. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening.

After incubation, the agent-biased binding between the IKAP polypeptide and one or more binding targets is detected by any convenient way. A difference in the binding affinity of the IKAP polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the

binding of the IKAP polypeptide to the IKAP binding target. Analogously, in the cell-based assay also described below, a difference in IKAP-dependent transcriptional activation in the presence and absence of an agent indicates the agent modulates IKAP function. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least a 90% difference.

5 The following experimental section and examples are offered by way of illustration and not by way of limitation.

EXAMPLES

1. Protocol for Cell-Based IKAP-NIK Interaction assay

10 IKAP has been identified as a NIK-interacting protein by coprecipitation assay :
293 cells are transfected with mammalian expression vectors encoding Flag-tagged NIK and Myc-tagged IKAP respectively. After 48 hours, cells are collected, washed twice with phosphate-buffered saline and lysed for 30 min at 4 °C in 0.5 ml of lysis buffer (50 mM HEPES pH 7.6, 100 mM NaCl, 1 % NP-40, 1 mM EDTA, 10 % glycerol) containing
15 phosphatase and protease inhibitors. Cellular debris are removed by centrifugation at 10,000 x g for 10 min twice. The NaCl concentration of the cell lysates is increased to 250 mM. The cell lysates are incubated for 1 hour on ice with 1 µg of anti-Flag monoclonal antibody or control mouse IgG1 antibody, and an additional hour at 4 °C with 15 µl of protein G-agarose beads. The beads are then collected, and washed four times with 1 ml of lysis buffer
20 containing 250 mM NaCl. The bound proteins are eluted, fractionated by SDS-PAGE and analyzed by western blotting using anti-Myc or anti-Flag polyclonal antibodies. The immunoblot is developed with horseradish peroxidase-coupled goat anti-rabbit immunoglobulin as secondary antibody and visualized using the Enhanced Chemoluminescence (ECL) Detection System.

2. Protocol for Cell-Based NF-κB Reporter Assay

25 IKAP can trans-activate NF-κB reporter constructs when overexpressed in 293 cells or HeLa cells. 293 cells are transfected using the calcium phosphate precipitation method with a plasmid encoding a 6 NF-κB-luciferase reporter construct and various
30 amounts of expression vector encoding IKAP. After 36-48 hours, cells are left untreated or treated with IL-1 (10-50 ng/ml) or TNF (50-100 ng) for 6 hours prior to harvest. Cells are

lysed and luciferase activity measured using the luciferase assay kit (Promega). The luciferase activity in each transfection is normalized by co-transfecting a pRSV- β gal control vector.

3. Protocol for high throughput in vitro IKAP-NIK binding assay.

5 A. Reagents:

- Neutralite Avidin: 20 μ g/ml in PBS.

- Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.

- Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM β -mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.

10 - ^{33}P IKAP polypeptide 10x stock: 10^{-8} - 10^{-6} M "cold" IKAP supplemented with 200,000-250,000 cpm of labeled IKAP (Beckman counter). Place in the 4°C microfridge during screening.

15 - Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM $NaVO_3$ (Sigma # S-6508) in 10 ml of PBS.

- NIK: 10^{-7} - 10^{-5} M biotinylated NIK in PBS.

B. Preparation of assay plates:

- Coat with 120 μ l of stock N-Avidin per well overnight at 4°C.

20 - Wash 2 times with 200 μ l PBS.

- Block with 150 μ l of blocking buffer.

- Wash 2 times with 200 μ l PBS.

C. Assay:

- Add 40 μ l assay buffer/well.

25 - Add 10 μ l compound or extract.

- Add 10 μ l ^{33}P -IKAP (20-25,000 cpm/0.1-10 pmoles/well = 10^{-9} - 10^{-7} M final conc).

- Shake at 25°C for 15 minutes.

- Incubate additional 45 minutes at 25°C.

- Add 40 μ M biotinylated NIK (0.1-10 pmoles/40 μ l in assay buffer)

30 - Incubate 1 hour at room temperature.

- Stop the reaction by washing 4 times with 200 μ M PBS.
- Add 150 μ M scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

- a. Non-specific binding
- b. Soluble (non-biotinylated NIK) at 80% inhibition.

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All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for

10 purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising SEQ ID NO:2 or a fragment thereof selected from the group consisting of: residues 1-10, 29-41, 75-87, 92-109, 132-141, 192-205, 258-269, 295-311, 316-330, 373-382, 403-422, 474-485, 561-576, 683-697, 768-777, 798-813, 1054-1067, 1181-1192, 1273-1282, 1283-1294, 1295-1312 and 1313-1332, wherein said domain has an IKAP activity selected from at least one of: a NIK-binding or binding inhibitory activity, an NF κ B activating or inhibitory activity and an IKAP-specific immunogenicity and/or antigenicity.

2. A recombinant nucleic acid comprising a coding region encoding a polypeptide according to claim 1 flanked by fewer than 2 kb of native flanking sequence.

3. A recombinant nucleic acid comprising a strand of SEQ ID NO:1 or of a fragment selected from the group consisting of nucleotides 1-47, 58-99, 95-138, 181-220, 261-299, 274-315, 351-389, 450-593, 524-546, 561-608, 689-727, 808-837 and 2910-2953, wherein the strand is flanked by fewer than 2 kb of native flanking sequence.

4. A cell comprising a nucleic acid according to claim 2 or 3.

5. A method of making an isolated polypeptide according to claim 1, said method comprising steps: introducing a recombinant nucleic acid encoding a polypeptide according to claim 1 into a host cell or cellular extract, incubating said host cell or extract under conditions whereby said nucleic acid is expressed as a transcript and said transcript is expressed as a translation product comprising said polypeptide, and isolating said translation product.

6. A method of screening for an agent which modulates the interaction of an IKAP polypeptide to a binding target, said method comprising the steps of:

incubating a mixture comprising:

an isolated polypeptide according to claim 1,

a binding target of said polypeptide, and

a candidate agent:

under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity;

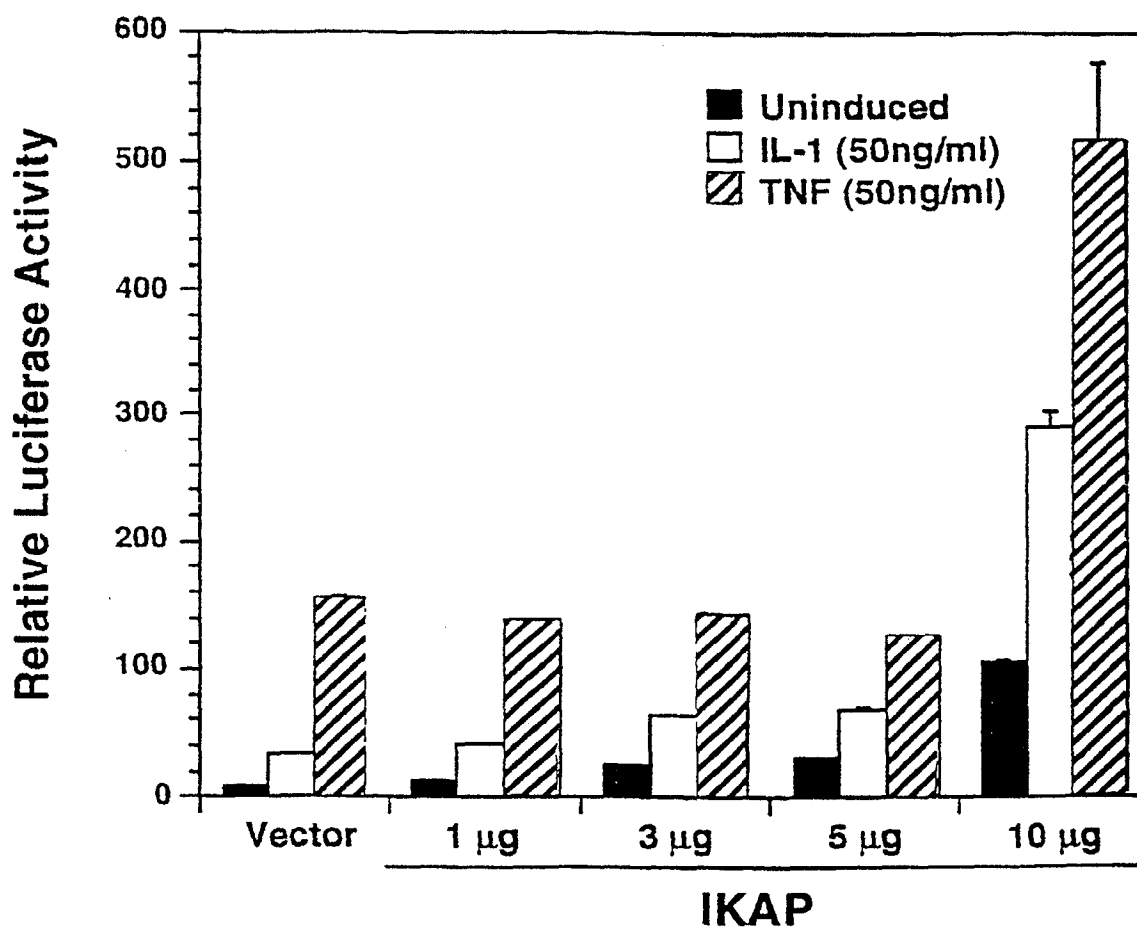
detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

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7. A method for modulating signal transduction in a cell, said method comprising the step of contacting the cell with an agent which modulates IKAP activity, wherein the agent is a nucleic acid according to claim 2 or 3.

10

FIG. 1



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cohen, Lucy

Baeuerle, Patrick

(ii) TITLE OF INVENTION: IKAP Proteins, Nucleic Acids and Methods

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SCIENCE & TECHNOLOGY LAW GROUP

(B) STREET: 75 DENISE DRIVE

(C) CITY: HILLSBOROUGH

(D) STATE: CALIFORNIA

(E) COUNTRY: USA

(F) ZIP: 94010

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME: OSMAN, RICHARD A

(B) REGISTRATION NUMBER: 36,627

(C) REFERENCE/DOCKET NUMBER: T97-011

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (650) 343-4341

(B) TELEFAX: (650) 343-4342

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3999 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..3996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG CGA AAT CTG AAA TTA TTT CGG ACC CTG GAG TTC AGG GAT ATT CAA	48
Met Arg Asn Leu Lys Leu Phe Arg Thr Leu Glu Phe Arg Asp Ile Gln	
1 5 10 15	
GGT CCA GGG AAT CCT CAG TGC TTC TCT CTC CGA ACT GAA CAG GGG ACG	96
Gly Pro Gly Asn Pro Gln Cys Phe Ser Leu Arg Thr Glu Gln Gly Thr	
20 25 30	
GTG CTC ATT GGT TCA GAA CAT GGC CTG ATA GAA GTA GAC CCT GTC TCA	144
Val Leu Ile Gly Ser Glu His Gly Leu Ile Glu Val Asp Pro Val Ser	
35 40 45	
AGA GAA GTG AAA AAT GAA GTT TCT TTG GTG GCA GAA GGC TTT CTT CCA	192
Arg Glu Val Lys Asn Glu Val Ser Leu Val Ala Glu Gly Phe Leu Pro	
50 55 60	
GAG GAT GGA AGT GGC CGC ATT GTT GGT GTT CAG GAC TTG CTG GAT CAG	240
Glu Asp Gly Ser Gly Arg Ile Val Gly Val Gln Asp Leu Leu Asp Gln	
65 70 75 80	

	GAG TCT GTG TGT GTG GCC ACA GCC TCT GGA GAC GTC ATA CTC TGC AGT	288
	Glu Ser Val Cys Val Ala Thr Ala Ser Gly Asp Val Ile Leu Cys Ser	
	85 90 95	
5	CTC AGC ACA CAA CAG CTG GAG TGT GTT GGG AGT GTA GCC AGT GGT ATC	336
	Leu Ser Thr Gln Gln Leu Glu Cys Val Gly Ser Val Ala Ser Gly Ile	
	100 105 110	
	TCT GTT ATG AGT TGG AGT CCT GAC CAA GAG CTG GTG CTT CTT GCC ACA	384
	Ser Val Met Ser Trp Ser Pro Asp Gln Glu Leu Val Leu Leu Ala Thr	
	115 120 125	
10	GGT CAA CAG ACC CTG ATT ATG ATG ACA AAA GAT TTT GAG CCA ATC CTG	432
	Gly Gln Gln Thr Leu Ile Met Met Thr Lys Asp Phe Glu Pro Ile Leu	
	130 135 140	
	GAG CAG CAG ATC CAT CAG GAT GAT TTT GGT GAA AGC AAG TTT ATC ACT	480
	Glu Gln Gln Ile His Gln Asp Asp Phe Gly Glu Ser Lys Phe Ile Thr	
	145 150 155 160	
15	GTT GGA TGG GGT AGG AAG GAG ACA CAG TTC CAT GGA TCA GAA GGC AGA	528
	Val Gly Trp Gly Arg Lys Glu Thr Gln Phe His Gly Ser Glu Gly Arg	
	165 170 175	
	CAA GCA GCT TTT CAG ATG CAA ATG CAT GAG TCT GCT TTG CCC TGG GAT	576
	Gln Ala Ala Phe Gln Met Gln Met His Glu Ser Ala Leu Pro Trp Asp	
	180 185 190	
20	GAC CAT AGA CCA CAA GTT ACC TGG CGG GGG GAT GGA CAG TTT TTT GCT	624
	Asp His Arg Pro Gln Val Thr Trp Arg Gly Asp Gly Gln Phe Phe Ala	
	195 200 205	
	GTG AGT GTT GTT TGC CCA GAA ACA GGG GCT CGG AAG GTC AGA GTG TGG	672
	Val Ser Val Val Cys Pro Glu Thr Gly Ala Arg Lys Val Arg Val Trp	
	210 215 220	
	AAC CGA GAG TTT GCT TTG CAG TCA ACC AGT GAG CCT GTG GCA GGA CTG	720
	Asn Arg Glu Phe Ala Leu Gln Ser Thr Ser Glu Pro Val Ala Gly Leu	
	225 230 235 240	
30	GGA CCA GCC CTG GCT TGG AAA CCC TCA GGC AGT TTG ATT GCA TCT ACA	768
	Gly Pro Ala Leu Ala Trp Lys Pro Ser Gly Ser Leu Ile Ala Ser Thr	
	245 250 255	
	CAA GAT AAA CCC AAC CAG CAG GAT ATT GTG TTT TTT GAG AAA AAT GGA	816
	Gln Asp Lys Pro Asn Gln Gln Asp Ile Val Phe Phe Glu Lys Asn Gly	
	260 265 270	
35	CTC CTT CAT GGA CAC TTT ACA CTT CCC TTC CTT AAA GAT GAG GTT AAG	864
	Leu Leu His Gly His Phe Thr Leu Pro Phe Leu Lys Asp Glu Val Lys	
	275 280 285	
	GTA AAT GAC TTG CTC TGG AAT GCA GAT TCC TCT GTG CTT GCA GTC CGG	912
	Val Asn Asp Leu Leu Trp Asn Ala Asp Ser Ser Val Leu Ala Val Arg	
	290 295 300	
	CTG GAA GAC CTT CAG AGA GAA AAA AGC TCC ATT CCG AAA ACC TGT GTT	960
	Leu Glu Asp Leu Gln Arg Glu Lys Ser Ser Ile Pro Lys Thr Cys Val	
	305 310 315 320	
45	CAG CTC TGG ACT GTT GGA AAC TAT CAC TGG TAT CTC AAG CAA AGT TTA	1008
	Gln Leu Trp Thr Val Gly Asn Tyr His Trp Tyr Leu Lys Gln Ser Leu	
	325 330 335	
	TCC TTC AGC ACC TGT GGG AAG AGC AAG ATT GTG TCT CTG ATG TGG GAC	1056
	Ser Phe Ser Thr Cys Gly Lys Ser Lys Ile Val Ser Leu Met Trp Asp	
	340 345 350	
50	CCT GTG ACC CCA TAC CGG CTG CAT GTT CTC TGT CAG GGC TGG CAT TAC	1104
	Pro Val Thr Pro Tyr Arg Leu His Val Leu Cys Gln Gly Trp His Tyr	
	355 360 365	
	CTC GCC TAT GAT TGG CAC TGG ACG ACT GAC CGG AGC GTG GGA GAT AAT	1152
	Leu Ala Tyr Asp Trp His Trp Thr Thr Asp Arg Ser Val Gly Asp Asn	
	370 375 380	

	TCA	AGT	GAC	TTG	TCC	AAT	GTG	GCT	GTC	ATT	GAT	GGA	AAC	AGG	GTG	TTG	1200
	Ser	Ser	Asp	Leu	Ser	Asn	Val	Ala	Val	Ile	Asp	Gly	Asn	Arg	Val	Leu	
	385					390					395					400	
	GTG	ACA	GTC	TTC	CGG	CAG	ACT	GTG	GTT	CCG	CCT	CCC	ATG	TGC	ACC	TAC	1248
5	Val	Thr	Val	Phe	Arg	Gln	Thr	Val	Val	Pro	Pro	Pro	Met	Cys	Thr	Tyr	
				405						410						415	
	CAA	CTG	CTG	TTC	CCA	CAC	CCT	GTG	AAT	CAA	GTC	ACA	TTC	TTA	GCA	CAC	1296
	Gln	Leu	Leu	Phe	Pro	His	Pro	Val	Asn	Gln	Val	Thr	Phe	Leu	Ala	His	
				420						425						430	
10	CCT	CAA	AAG	AGT	AAT	GAC	CTT	GCT	GTT	CTA	GAT	GCC	AGT	AAC	CAG	ATT	1344
	Pro	Gln	Lys	Ser	Asn	Asp	Leu	Ala	Val	Leu	Asp	Ala	Ser	Asn	Gln	Ile	
			435						440							445	
	TCT	GTT	TAT	AAA	TGT	GGT	GAT	TGT	CCA	AGT	GCT	GAC	CCT	ACA	GTG	AAA	1392
	Ser	Val	Tyr	Lys	Cys	Gly	Asp	Cys	Pro	Ser	Ala	Asp	Pro	Thr	Val	Lys	
			450				455					460					
15	CTG	GGA	GCT	GTG	GGT	GGA	AGT	GGA	TTT	AAA	GTT	TGC	CTT	AGA	ACT	CCT	1440
	Leu	Gly	Ala	Val	Gly	Gly	Ser	Gly	Phe	Lys	Val	Cys	Leu	Arg	Thr	Pro	
				465			470					475				480	
	CAT	TTG	GAA	AAG	AGA	TAC	AAA	ATC	CAG	TTT	GAG	AAT	AAT	GAA	GAT	CAA	1488
	His	Leu	Glu	Lys	Arg	Tyr	Lys	Ile	Gln	Phe	Glu	Asn	Asn	Glu	Asp	Gln	
20				485						490						495	
	GAT	GTA	AAC	CCG	CTG	AAA	CTA	GGC	CTT	CTC	ACT	TGG	ATT	GAA	GAA	GAC	1536
	Asp	Val	Asn	Pro	Leu	Lys	Leu	Gly	Leu	Leu	Thr	Trp	Ile	Glu	Glu	Asp	
				500						505						510	
25	GTC	TTC	CTG	GCT	GTA	AGC	CAC	AGT	GAG	TTC	AGC	CCC	CGG	TCT	GTC	ATT	1584
	Val	Phe	Leu	Ala	Val	Ser	His	Ser	Glu	Phe	Ser	Pro	Arg	Ser	Val	Ile	
				515					520							525	
	CAC	CAT	TTG	ACT	GCA	GCT	TCT	TCT	GAG	ATG	GAT	GAA	GAG	CAT	GGA	CAG	1632
	His	His	Leu	Thr	Ala	Ala	Ser	Ser	Glu	Met	Asp	Glu	Glu	His	Gly	Gln	
				530				535								540	
30	CTC	AAT	GTC	AGT	TCA	TCT	GCA	GCG	GTG	GAT	GGG	GTC	ATA	ATC	AGT	CTA	1680
	Leu	Asn	Val	Ser	Ser	Ser	Ala	Ala	Val	Asp	Gly	Val	Ile	Ile	Ser	Leu	
				545			550				555					560	
	TGT	TGC	AAT	TCC	AAG	ACC	AAG	TCA	GTA	GTA	TTA	CAG	CTG	GCT	GAT	GGC	1728
35	Cys	Cys	Asn	Ser	Lys	Thr	Lys	Ser	Val	Val	Leu	Gln	Leu	Ala	Asp	Gly	
				565						570						575	
	CAG	ATA	TTT	AAG	TAC	CTT	TGG	GAG	TCA	CCT	TCT	CTG	GCT	ATT	AAA	CCA	1776
	Gln	Ile	Phe	Lys	Tyr	Leu	Trp	Glu	Ser	Pro	Ser	Leu	Ala	Ile	Lys	Pro	
				580					585							590	
40	TGG	AAG	AAC	TCT	GGT	GGA	TTT	CCT	GTT	CGG	TTT	CCT	TAT	CCA	TGC	ACC	1824
	Trp	Lys	Asn	Ser	Gly	Gly	Phe	Pro	Val	Arg	Phe	Pro	Tyr	Pro	Cys	Thr	
				595				600								605	
	CAG	ACC	GAA	TTG	GCC	ATG	ATT	GGA	GAA	GAG	GAA	TGT	GTC	CTT	GGT	CTG	1872
	Gln	Thr	Glu	Leu	Ala	Met	Ile	Gly	Glu	Glu	Glu	Cys	Val	Leu	Gly	Leu	
				610				615								620	
45	ACT	GAC	AGG	TGT	CGC	TTT	TTC	ATC	AAT	GAC	ATT	GAG	GTT	GCG	TCA	AAT	1920
	Thr	Asp	Arg	Cys	Arg	Phe	Phe	Ile	Asn	Asp	Ile	Glu	Val	Ala	Ser	Asn	
				625			630					635				640	
	ATC	ACG	TCA	TTT	GCA	GTA	TAT	GAT	GAG	TTT	TTA	TTG	TTG	ACA	ACC	CAT	1968
	Ile	Thr	Ser	Phe	Ala	Val	Tyr	Asp	Glu	Phe	Leu	Leu	Leu	Thr	Thr	His	
50				645							650					655	
	TCC	CAT	ACC	TGC	CAG	TGT	TTT	TGC	CTG	AGG	GAT	GCT	TCA	TTT	AAA	ACA	2016
	Ser	His	Thr	Cys	Gln	Cys	Phe	Cys	Leu	Arg	Asp	Ala	Ser	Phe	Lys	Thr	
				660						665						670	
55	TTA	CAG	GCC	GGC	CTG	AGC	AGC	AAT	CAT	GTG	TCC	CAT	GGG	GAA	GTT	CTG	2064
	Leu	Gln	Ala	Gly	Leu	Ser	Ser	Asn	His	Val	Ser	His	Gly	Glu	Val	Leu	
				675				680								685	

	CGG AAA GTG GAG AGG GGT TCA CGG ATT GTC ACT GTT GTG CCC CAG GAC	2112
	Arg Lys Val Glu Arg Gly Ser Arg Ile Val Thr Val Val Pro Gln Asp	
	690 695 700	
5	ACA AAG CTT GTA TTA CAG ATG CCA AGG GGA AAC TTA GAA GTT GTT CAT	2160
	Thr Lys Leu Val Leu Gln Met Pro Arg Gly Asn Leu Glu Val Val His	
	705 710 715 720	
	CAT CGA GCC CTG GTT TTA GCT CAG ATT CGG AAG TGG TTG GAC AAA CTT	2208
	His Arg Ala Leu Val Leu Ala Gln Ile Arg Lys Trp Leu Asp Lys Leu	
	725 730 735	
10	ATG TTT AAA GAG GCA TTT GAA TGC ATG AGA AAG CTG AGA ATC AAT CTC	2256
	Met Phe Lys Glu Ala Phe Glu Cys Met Arg Lys Leu Arg Ile Asn Leu	
	740 745 750	
	AAT CCG ATT TAT GAT CAT AAC CCT AAG GTG TTT CTT GGA AAT GTG GAA	2304
	Asn Pro Ile Tyr Asp His Asn Pro Lys Val Phe Leu Gly Asn Val Glu	
	755 760 765	
15	ACC TTC ATT AAA CAG ATA GAT TCT GTG AAT CAT ATT AAC TTG TTT TTT	2352
	Thr Phe Ile Lys Gln Ile Asp Ser Val Asn His Ile Asn Leu Phe Phe	
	770 775 780	
20	ACA GAA TTG AAA GAA GAA GAT GTC ACG AAG ACC ATG TAC CCT GCA CCA	2400
	Thr Glu Leu Lys Glu Glu Asp Val Thr Lys Thr Met Tyr Pro Ala Pro	
	785 790 795 800	
	GTT ACC AGC AGT GTC TAC CTG TCC AGG GAT CCT GAC GGG AAT AAA ATA	2448
	Val Thr Ser Ser Val Tyr Leu Ser Arg Asp Pro Asp Gly Asn Lys Ile	
	805 810 815	
25	GAC CTT GTC TGC GAT GCT ATG AGA GCA GTC ATG GAG AGC ATA AAT CCT	2496
	Asp Leu Val Cys Asp Ala Met Arg Ala Val Met Glu Ser Ile Asn Pro	
	820 825 830	
	CAT AAA TAC TGC CTA TCC ATA CTT ACA TCT CAT GTA AAG AAG ACA ACC	2544
	His Lys Tyr Cys Leu Ser Ile Leu Thr Ser His Val Lys Lys Thr Thr	
	835 840 845	
30	CCA GAA CTG GAA ATT GTA CTG CAA AAA GTA CAC GAG CTT CAA GGA AAT	2592
	Pro Glu Leu Glu Ile Val Leu Gln Lys Val His Glu Leu Gln Gly Asn	
	850 855 860	
35	GCT CCC TCT GAT CCT GAT GCT GTG AGT GCT GAA GAG GCC TTG AAA TAT	2640
	Ala Pro Ser Asp Pro Asp Ala Val Ser Ala Glu Ala Leu Lys Tyr	
	865 870 875 880	
	TTG CTG CAT CTG GTA GAT GTT AAT GAA TTA TAT GAT CAT TCT CTT GGC	2688
	Leu Leu His Leu Val Asp Val Asn Glu Leu Tyr Asp His Ser Leu Gly	
	885 890 895	
40	ACC TAT GAC TTT GAT TTG GTC CTC ATG GTA GCT GAG AAG TCA CAG AAG	2736
	Thr Tyr Asp Phe Asp Leu Val Leu Met Val Ala Glu Lys Ser Gln Lys	
	900 905 910	
	GAT CCC AAA GAA TAT CTT CCA TTT CTT AAT ACA CTT AAG AAA ATG GAA	2784
	Asp Pro Lys Glu Tyr Leu Pro Phe Leu Asn Thr Leu Lys Lys Met Glu	
	915 920 925	
45	ACT AAT TAT CAG CGG TTT ACT ATA GAC AAA TAC TTG AAA CGA TAT GAA	2832
	Thr Asn Tyr Gln Arg Phe Thr Ile Asp Lys Tyr Leu Lys Arg Tyr Glu	
	930 935 940	
50	AAA GCC ATT GGC CAC CTC AGC AAA TGT GGA CCT GAG TAC TTC CCA GAA	2880
	Lys Ala Ile Gly His Leu Ser Lys Cys Gly Pro Glu Tyr Phe Pro Glu	
	945 950 955 960	
	TGC TTA AAC TTG ATA AAA GAT AAA AAC TTG TAT AAC GAA GCT CTG AAG	2928
	Cys Leu Asn Leu Ile Lys Asp Lys Asn Leu Tyr Asn Glu Ala Leu Lys	
	965 970 975	
55	TTA TAT TCA CCA AGC TCA CAA CAG TAC CAG GAT ATC AGC ATT GCT TAT	2976
	Leu Tyr Ser Pro Ser Ser Gln Gln Tyr Gln Asp Ile Ser Ile Ala Tyr	
	980 985 990	

	GGG GAG CAC CTG ATG CAG GAG CAC ATG TAT GAG CCA GCG GGG CTC ATG	3024
	Gly Glu His Leu Met Gln Glu His Met Tyr Glu Pro Ala Gly Leu Met	
	995 1000 1005	
	TTT GCC CGT TGC GGT GCC CAC GAG AAA GCT CTC TCA GCC TTT CTC ACA	3072
	Phe Ala Arg Cys Gly Ala His Glu Lys Ala Leu Ser Ala Phe Leu Thr	
5	1010 1015 1020	
	TGT GGC AAC TGG AAG CAA GCC CTC TGT GTG GCA GCC CAG CTT AAC TTT	3120
	Cys Gly Asn Trp Lys Gln Ala Leu Cys Val Ala Ala Gln Leu Asn Phe	
	1025 1030 1035 1040	
10	ACC AAA GAC CAG CTG GTG GGC CTC GGC AGA ACT CTG GCA GGA AAG CTG	3168
	Thr Lys Asp Gln Leu Val Gly Leu Gly Arg Thr Leu Ala Gly Lys Leu	
	1045 1050 1055	
	GTT GAG CAG AGG AAG CAC ATT GAT GCG GCC ATG GTT TTG GAA GAG TGT	3216
	Val Glu Gln Arg Lys His Ile Asp Ala Ala Met Val Leu Glu Glu Cys	
	1060 1065 1070	
15	GCC CAG GAT TAT GAA GAA GCT GTG CTC TTG CTG TTA GAA GGA GCT GCC	3264
	Ala Gln Asp Tyr Glu Glu Ala Val Leu Leu Leu Leu Glu Gly Ala Ala	
	1075 1080 1085	
	TGG GAA GAA GCT TTG AGG CTG GTA TAC AAA TAT AAC AGA CTG GAT ATT	3312
	Trp Glu Glu Ala Leu Arg Leu Val Tyr Lys Tyr Asn Arg Leu Asp Ile	
20	1090 1095 1100	
	ATA GAA ACC AAC GTA AAG CCT TCC ATT TTA GAA GCC CAG AAA AAT TAT	3360
	Ile Glu Thr Asn Val Lys Pro Ser Ile Leu Glu Ala Gln Lys Asn Tyr	
	1105 1110 1115 1120	
25	ATG GCA TTT CTG GAC TCT CAG ACA GCC ACA TTC AGT CGC CAC AAG AAA	3408
	Met Ala Phe Leu Asp Ser Gln Thr Ala Thr Phe Ser Arg His Lys Lys	
	1125 1130 1135	
	CGT TTA TTG GTA GTT CGA GAG CTC AAG GAG CAA GCC CAG CAG GCA GGT	3456
	Arg Leu Leu Val Val Arg Glu Leu Lys Glu Gln Ala Gln Gln Ala Gly	
	1140 1145 1150	
30	CTG GAT GAT GAG GTA CCC CAC GGG CAA GAG TCA GAC CTC TTC TCT GAA	3504
	Leu Asp Asp Glu Val Pro His Gly Gln Glu Ser Asp Leu Phe Ser Glu	
	1155 1160 1165	
	ACT AGC AGT GTC GTG AGT GGC AGT GAG ATG AGT GGC AAA TAC TCC CAT	3552
	Thr Ser Ser Val Val Ser Gly Ser Glu Met Ser Gly Lys Tyr Ser His	
35	1170 1175 1180	
	AGT AAC TCC AGG ATA TCA GCG AGA TCA TCC AAG AAT CGC CGA AAA GCG	3600
	Ser Asn Ser Arg Ile Ser Ala Arg Ser Ser Lys Asn Arg Arg Lys Ala	
	1185 1190 1195 1200	
40	GAG CGG AAG AAG CAC AGC CTC AAA GAA GGC ACT CCG CTG GAG GAC CTG	3648
	Glu Arg Lys Lys His Ser Leu Lys Glu Gly Ser Pro Leu Glu Asp Leu	
	1205 1210 1215	
	GCC CTC CTG GAG GCA CTG AGT GAA GTG GTG CAG AAC ACT GAA AAC CTG	3696
	Ala Leu Leu Glu Ala Leu Ser Glu Val Val Gln Asn Thr Glu Asn Leu	
	1220 1225 1230	
45	AAA GAT GAA GTA TAC CAT ATT TTA AAG GTA CTC TTT CTC TTT GAG TTT	3744
	Lys Asp Glu Val Tyr His Ile Leu Lys Val Leu Phe Leu Phe Glu Phe	
	1235 1240 1245	
	GAT GAA CAA GGA AGG GAA TTA CAG AAG GCC TTT GAA GAT ACG CTG CAG	3792
	Asp Glu Gln Gly Arg Glu Leu Gln Lys Ala Phe Glu Asp Thr Leu Gln	
50	1250 1255 1260	
	TTG ATG GAA AGG TCA CTT CCA GAA ATT TGG ACT CTT ACT TAC CAG CAG	3840
	Leu Met Glu Arg Ser Leu Pro Glu Ile Trp Thr Leu Thr Tyr Gln Gln	
	1265 1270 1275 1280	
55	AAT TCA GCT ACC CCG GTT CTA GGT CCC AAT TCT ACT GCA AAT AGT ATC	3888
	Asn Ser Ala Thr Pro Val Leu Gly Pro Asn Ser Thr Ala Asn Ser Ile	
	1285 1290 1295	

ATG GCA TCT TAT CAG CAA CAG AAG ACT TCG GTT CCT GTT CTT GAT GCT 3936
 Met Ala Ser Tyr Gln Gln Gln Lys Thr Ser Val Pro Val Leu Asp Ala
 1300 1305 1310
 GAG CTT TTT ATA CCA CCA AAG ATC AAC AGA AGA ACC CAG TGG AAG CTG 3984
 Glu Leu Phe Ile Pro Pro Lys Ile Asn Arg Arg Thr Gln Trp Lys Leu
 1315 1320 1325
 AGC CTG CTA GAC TGA 3999
 Ser Leu Leu Asp
 1330

10 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1332 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Asn Leu Lys Leu Phe Arg Thr Leu Glu Phe Arg Asp Ile Gln
 1 5 10 15
 Gly Pro Gly Asn Pro Gln Cys Phe Ser Leu Arg Thr Glu Gln Gly Thr
 20 20 25 30
 Val Leu Ile Gly Ser Glu His Gly Leu Ile Glu Val Asp Pro Val Ser
 35 40 45
 Arg Glu Val Lys Asn Glu Val Ser Leu Val Ala Glu Gly Phe Leu Pro
 50 55 60
 Glu Asp Gly Ser Gly Arg Ile Val Gly Val Gln Asp Leu Leu Asp Gln
 25 65 70 75 80
 Glu Ser Val Cys Val Ala Thr Ala Ser Gly Asp Val Ile Leu Cys Ser
 85 90 95
 Leu Ser Thr Gln Gln Leu Glu Cys Val Gly Ser Val Ala Ser Gly Ile
 30 100 105 110
 Ser Val Met Ser Trp Ser Pro Asp Gln Glu Leu Val Leu Leu Ala Thr
 115 120 125
 Gly Gln Gln Thr Leu Ile Met Met Thr Lys Asp Phe Glu Pro Ile Leu
 130 135 140
 Glu Gln Gln Ile His Gln Asp Asp Phe Gly Glu Ser Lys Phe Ile Thr
 35 145 150 155 160
 Val Gly Trp Gly Arg Lys Glu Thr Gln Phe His Gly Ser Glu Gly Arg
 165 170 175
 Gln Ala Ala Phe Gln Met Gln Met His Glu Ser Ala Leu Pro Trp Asp
 40 180 185 190
 Asp His Arg Pro Gln Val Thr Trp Arg Gly Asp Gly Gln Phe Phe Ala
 195 200 205
 Val Ser Val Val Cys Pro Glu Thr Gly Ala Arg Lys Val Arg Val Trp
 210 215 220
 Asn Arg Glu Phe Ala Leu Gln Ser Thr Ser Glu Pro Val Ala Gly Leu
 45 225 230 235 240
 Gly Pro Ala Leu Ala Trp Lys Pro Ser Gly Ser Leu Ile Ala Ser Thr
 245 250 255
 Gln Asp Lys Pro Asn Gln Gln Asp Ile Val Phe Phe Glu Lys Asn Gly
 50 260 265 270
 Leu Leu His Gly His Phe Thr Leu Pro Phe Leu Lys Asp Glu Val Lys
 275 280 285
 Val Asn Asp Leu Leu Trp Asn Ala Asp Ser Ser Val Leu Ala Val Arg
 290 295 300
 55 Leu Glu Asp Leu Gln Arg Glu Lys Ser Ser Ile Pro Lys Thr Cys Val
 305 310 315 320

	Gln	Leu	Trp	Thr	Val	Gly	Asn	Tyr	His	Trp	Tyr	Leu	Lys	Gln	Ser	Leu
					325					330					335	
	Ser	Phe	Ser	Thr	Cys	Gly	Lys	Ser	Lys	Ile	Val	Ser	Leu	Met	Trp	Asp
					340					345					350	
5	Pro	Val	Thr	Pro	Tyr	Arg	Leu	His	Val	Leu	Cys	Gln	Gly	Trp	His	Tyr
					355					360					365	
	Leu	Ala	Tyr	Asp	Trp	His	Trp	Thr	Thr	Asp	Arg	Ser	Val	Gly	Asp	Asn
					370					375				380		
	Ser	Ser	Asp	Leu	Ser	Asn	Val	Ala	Val	Ile	Asp	Gly	Asn	Arg	Val	Leu
						390					395					400
10	Val	Thr	Val	Phe	Arg	Gln	Thr	Val	Val	Pro	Pro	Pro	Met	Cys	Thr	Tyr
					405					410						415
	Gln	Leu	Leu	Phe	Pro	His	Pro	Val	Asn	Gln	Val	Thr	Phe	Leu	Ala	His
					420					425					430	
15	Pro	Gln	Lys	Ser	Asn	Asp	Leu	Ala	Val	Leu	Asp	Ala	Ser	Asn	Gln	Ile
					435					440					445	
	Ser	Val	Tyr	Lys	Cys	Gly	Asp	Cys	Pro	Ser	Ala	Asp	Pro	Thr	Val	Lys
						450										
	Leu	Gly	Ala	Val	Gly	Gly	Ser	Gly	Phe	Lys	Val	Cys	Leu	Arg	Thr	Pro
					465											480
20	His	Leu	Glu	Lys	Arg	Tyr	Lys	Ile	Gln	Phe	Glu	Asn	Asn	Glu	Asp	Gln
					485						490					495
	Asp	Val	Asn	Pro	Leu	Lys	Leu	Gly	Leu	Leu	Thr	Trp	Ile	Glu	Glu	Asp
					500					505					510	
25	Val	Phe	Leu	Ala	Val	Ser	His	Ser	Glu	Phe	Ser	Pro	Arg	Ser	Val	Ile
					515					520						
	His	His	Leu	Thr	Ala	Ala	Ser	Ser	Glu	Met	Asp	Glu	Glu	His	Gly	Gln
					530					535						
	Leu	Asn	Val	Ser	Ser	Ser	Ala	Ala	Val	Asp	Gly	Val	Ile	Ile	Ser	Leu
					545						550					560
30	Cys	Cys	Asn	Ser	Lys	Thr	Lys	Ser	Val	Val	Leu	Gln	Leu	Ala	Asp	Gly
					565						570					575
	Gln	Ile	Phe	Lys	Tyr	Leu	Trp	Glu	Ser	Pro	Ser	Leu	Ala	Ile	Lys	Pro
					580					585						590
35	Trp	Lys	Asn	Ser	Gly	Gly	Phe	Pro	Val	Arg	Phe	Pro	Tyr	Pro	Cys	Thr
					595					600						
	Gln	Thr	Glu	Leu	Ala	Met	Ile	Gly	Glu	Glu	Glu	Cys	Val	Leu	Gly	Leu
					610											
	Thr	Asp	Arg	Cys	Arg	Phe	Phe	Ile	Asn	Asp	Ile	Glu	Val	Ala	Ser	Asn
					625											640
40	Ile	Thr	Ser	Phe	Ala	Val	Tyr	Asp	Glu	Phe	Leu	Leu	Leu	Thr	Thr	His
					645						650					655
	Ser	His	Thr	Cys	Gln	Cys	Phe	Cys	Leu	Arg	Asp	Ala	Ser	Phe	Lys	Thr
					660					665						670
45	Leu	Gln	Ala	Gly	Leu	Ser	Ser	Asn	His	Val	Ser	His	Gly	Glu	Val	Leu
					675					680						
	Arg	Lys	Val	Glu	Arg	Gly	Ser	Arg	Ile	Val	Thr	Val	Val	Pro	Gln	Asp
					690											
	Thr	Lys	Leu	Val	Leu	Gln	Met	Pro	Arg	Gly	Asn	Leu	Glu	Val	Val	His
					705											720
50	His	Arg	Ala	Leu	Val	Leu	Ala	Gln	Ile	Arg	Lys	Trp	Leu	Asp	Lys	Leu
					725											735
	Met	Phe	Lys	Glu	Ala	Phe	Glu	Cys	Met	Arg	Lys	Leu	Arg	Ile	Asn	Leu
					740											
	Asn	Pro	Ile	Tyr	Asp	His	Asn	Pro	Lys	Val	Phe	Leu	Gly	Asn	Val	Glu
					755											
55	Thr	Phe	Ile	Lys	Gln	Ile	Asp	Ser	Val	Asn	His	Ile	Asn	Leu	Phe	Phe

	770					775						780					
	Thr	Glu	Leu	Lys	Glu	Glu	Asp	Val	Thr	Lys	Thr	Met	Tyr	Pro	Ala	Pro	
	785					790					795					800	
	Val	Thr	Ser	Ser	Val	Tyr	Leu	Ser	Arg	Asp	Pro	Asp	Gly	Asn	Lys	Ile	
					805					810					815		
5	Asp	Leu	Val	Cys	Asp	Ala	Met	Arg	Ala	Val	Met	Glu	Ser	Ile	Asn	Pro	
					820					825					830		
	His	Lys	Tyr	Cys	Leu	Ser	Ile	Leu	Thr	Ser	His	Val	Lys	Lys	Thr	Thr	
					835				840					845			
10	Pro	Glu	Leu	Glu	Ile	Val	Leu	Gln	Lys	Val	His	Glu	Leu	Gln	Gly	Asn	
					850				855					860			
	Ala	Pro	Ser	Asp	Pro	Asp	Ala	Val	Ser	Ala	Glu	Glu	Ala	Leu	Lys	Tyr	
	865					870					875					880	
	Leu	Leu	His	Leu	Val	Asp	Val	Asn	Glu	Leu	Tyr	Asp	His	Ser	Leu	Gly	
					885						890					895	
15	Thr	Tyr	Asp	Phe	Asp	Leu	Val	Leu	Met	Val	Ala	Glu	Lys	Ser	Gln	Lys	
					900					905					910		
	Asp	Pro	Lys	Glu	Tyr	Leu	Pro	Phe	Leu	Asn	Thr	Leu	Lys	Lys	Met	Glu	
					915					920				925			
20	Thr	Asn	Tyr	Gln	Arg	Phe	Thr	Ile	Asp	Lys	Tyr	Leu	Lys	Arg	Tyr	Glu	
						930				935				940			
	Lys	Ala	Ile	Gly	His	Leu	Ser	Lys	Cys	Gly	Pro	Glu	Tyr	Phe	Pro	Glu	
	945					950					955					960	
	Cys	Leu	Asn	Leu	Ile	Lys	Asp	Lys	Asn	Leu	Tyr	Asn	Glu	Ala	Leu	Lys	
					965						970					975	
25	Leu	Tyr	Ser	Pro	Ser	Ser	Gln	Gln	Tyr	Gln	Asp	Ile	Ser	Ile	Ala	Tyr	
					980					985					990		
	Gly	Glu	His	Leu	Met	Gln	Glu	His	Met	Tyr	Glu	Pro	Ala	Gly	Leu	Met	
					995				1000					1005			
30	Phe	Ala	Arg	Cys	Gly	Ala	His	Glu	Lys	Ala	Leu	Ser	Ala	Phe	Leu	Thr	
					1010				1015					1020			
	Cys	Gly	Asn	Trp	Lys	Gln	Ala	Leu	Cys	Val	Ala	Ala	Gln	Leu	Asn	Phe	
	1025					1030					1035					1040	
	Thr	Lys	Asp	Gln	Leu	Val	Gly	Leu	Gly	Arg	Thr	Leu	Ala	Gly	Lys	Leu	
					1045					1050						1055	
35	Val	Glu	Gln	Arg	Lys	His	Ile	Asp	Ala	Ala	Met	Val	Leu	Glu	Glu	Cys	
					1060					1065					1070		
	Ala	Gln	Asp	Tyr	Glu	Glu	Ala	Val	Leu	Leu	Leu	Leu	Glu	Gly	Ala	Ala	
					1075				1080					1085			
40	Trp	Glu	Glu	Ala	Leu	Arg	Leu	Val	Tyr	Lys	Tyr	Asn	Arg	Leu	Asp	Ile	
					1090				1095					1100			
	Ile	Glu	Thr	Asn	Val	Lys	Pro	Ser	Ile	Leu	Glu	Ala	Gln	Lys	Asn	Tyr	
	1105					1110					1115					1120	
	Met	Ala	Phe	Leu	Asp	Ser	Gln	Thr	Ala	Thr	Phe	Ser	Arg	His	Lys	Lys	
					1125					1130						1135	
45	Arg	Leu	Leu	Val	Val	Arg	Glu	Leu	Lys	Glu	Gln	Ala	Gln	Gln	Ala	Gly	
					1140					1145					1150		
	Leu	Asp	Asp	Glu	Val	Pro	His	Gly	Gln	Glu	Ser	Asp	Leu	Phe	Ser	Glu	
					1155				1160					1165			
50	Thr	Ser	Ser	Val	Val	Ser	Gly	Ser	Glu	Met	Ser	Gly	Lys	Tyr	Ser	His	
					1170				1175					1180			
	Ser	Asn	Ser	Arg	Ile	Ser	Ala	Arg	Ser	Ser	Lys	Asn	Arg	Arg	Lys	Ala	
	1185					1190					1195					1200	
	Glu	Arg	Lys	Lys	His	Ser	Leu	Lys	Glu	Gly	Ser	Pro	Leu	Glu	Asp	Leu	
					1205					1210					1215		
55	Ala	Leu	Leu	Glu	Ala	Leu	Ser	Glu	Val	Gln	Asn	Thr	Glu	Asn	Leu		
					1220				1225						1230		

Lys Asp Glu Val Tyr His Ile Leu Lys Val Leu Phe Leu Phe Glu Phe
 1235 1240 1245
 Asp Glu Gln Gly Arg Glu Leu Gln Lys Ala Phe Glu Asp Thr Leu Gln
 1250 1255 1260
 5 Leu Met Glu Arg Ser Leu Pro Glu Ile Trp Thr Leu Thr Tyr Gln Gln
 1265 1270 1275 1280
 Asn Ser Ala Thr Pro Val Leu Gly Pro Asn Ser Thr Ala Asn Ser Ile
 1285 1290 1295
 Met Ala Ser Tyr Gln Gln Gln Lys Thr Ser Val Pro Val Leu Asp Ala
 1300 1305 1310
 10 Glu Leu Phe Ile Pro Pro Lys Ile Asn Arg Arg Thr Gln Trp Lys Leu
 1315 1320 1325
 Ser Leu Leu Asp
 1330

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/24396

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350; 435/6, 7.1, 7.21, 69.1, 320.1, 325, 252.3, 254.11; 436/501; 536, 23.1, 23.5, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAPLUS, EMBASE, WPIDS, GENBANK

search terms: ikap, l cohen, p baeuerle

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank, National Library of Medicine, Bethesda,	2, 4
--	Maryland USA, Accession Number H19711, HILLIER et al.,	----
Y	yn60b07.rl Homo sapiens cDNA clone 172789 5'. 03 July 1995.	1, 5
X	Database GenBank, National Library of Medicine, Bethesda,	2, 4
--	Maryland USA, Accession Number N31333, HILLIER et al.,	---
Y	yx54c03.rl Homo sapiens cDNA clone 265540 5'. 10 January 1996.	1, 5
X	Database Genbank, National Library of Medicine, Bethesda,	2, 4
--	Maryland USA, Accession Number H15327, HILLIER et al.,	----
Y	ym28d08.rl Homo sapiens cDNA clone 49526 5'. 27 June 1995.	1, 5

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 FEBRUARY 1999

Date of mailing of the international search report

16 FEB 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

CLAIRE M. KAUFMAN

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24396

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	Database GenBank, National Library of Medicine, Bethesda, Maryland USA, Accession Number AA478901, HILLIER et al., zv20c02.rl Soares NhHMPu S1 Homo sapiens cDNA clone 754178 5'. 08 August 1997.	2, 4 ---- 1, 5
X -- Y	Database GenBank, National Library of Medicine, Bethesda, Maryland USA, Accession Number AA324126, HILLIER et al., EST27019 Cerebellum II Homo sapiens cDNA 5' end. 20 April 1997.	2, 4 ---- 1, 5
Y	WO 94/01548 A2 (MEDICAL RESEARCH COUNCIL) 20 January 1994, see entire document, especially claims 15 and 16, and page 10 line 37 through page 11 line 15.	1, 5